(51) International Patent Classification 6 :		(11) International Publication Number: **WO 97/36932**
C07K 19/00, A61K 47/48, 39/395, C07K 14/31	A1	(43) International Publication Date: 9 October 1997 (09.10.97)

(21) International Application Number: PCT/SE97/00537

(22) International Filing Date: 26 March 1997 (26.03.97)

(30) Priority Data:
9601245-5   29 March 1996 (29.03.96)   SE
08/695,692   12 August 1996 (12.08.96)   US

(71) Applicant (for all designated States except US): PHARMACIA & UPJOHN AB [SE/SE]; S-112 87 Stockholm (SE).

(72) Inventors; and
(75) Inventors/Applicants (for US only): ANTONSSON, Per [SE/SE]; Stenåldersvägen 63, S-222 54 Lund (SE). HANSSON, Johan [SE/SE]; Juryvägen 13, S-226 57 Lund (SE). BJÖRK, Per [SE/SE]; Sigridsgatan 31, S-256 56 Helsingborg (SE). DOHLSTEN, Mikael [SE/SE]; Gerdagatan 12B, S-223 62 Lund (SE). KALLAND, Terje [NO/IT]; Viale, 26/3, I-20020 Arese (IT). ABRAHMSÉN, Lars [SE/SE]; Lillängsgatan 28, S-168 58 Bromma (SE). FORSBERG, Göran [SE/SE]; Kulgränden 15A, S-226 49 Lund (SE).

(74) Agents: BERGANDER, Håkan et al.; Pharmacia & Upjohn AB, Patent Dept., S-751 82 Uppsala (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

(54) Title: MODIFIED/CHIMERIC SUPERANTIGENS AND THEIR USE
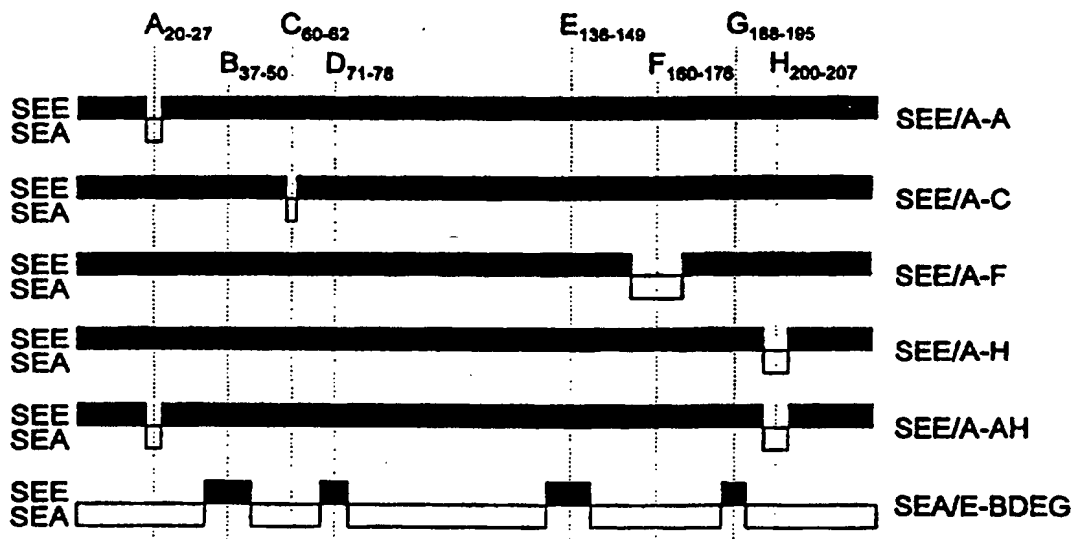
(57) Abstract

A conjugate between a target-seeking moiety and a modified superantigen, characterized in that the superantigen is a wild-type superantigen (SA I) in which an amino acid residue in a superantigen region (region I) determining binding to TCR, preferably TCRVβ, and T cell activation have been replaced by another amino acid residue while retaining the ability to activate a subset of T cells. In a preferred embodiment the modified superantigen is a chimer between at least two wild-type superantigens (SA I, SA II etc.) characterized in that one or more amino acid residues in a region determining binding to TCR and T cell activation have been interchanged between various wild-type superantigens. A therapeutic method making use of modified/chimeric superantigens as defined in the preceding paragraphs. An antibody preparation in which the cysteine residues that provide for interchain disulfide bonds have been mutated so as to forbid interchain disulfide bridges, preferably to serine residues, for use as a pharmaceutical.

MODIFIED/CHIMERIC SUPERANTIGENS AND THEIR USE

This application claims priority from Swedish Patent
Application No 9601245-5, which was filed March 29, 1996, and
5 from US application 08/695,692, which was filed August 12,
1996, both of which are incorporated herein by reference.

Field of the invention

The present invention relates to functionally active modified
10 superantigens which are wild-type superantigens (SA I) in which
one or more amino acid residues have been substituted while
maintaining superantigen function. In case one or more of the
substituting residues (or a conserved amino acid residue
thereof) occur in the corresponding positions in another wild-
15 type superantigen (SA II), the modified superantigen is called
a chimera (sometimes hybrid). Chimeric superantigens thus will
contain part sequences/regions deriving from at least two
different wild-type superantigens.

By the term "corresponding" is meant that residues, part
20 sequences and regions replacing each other have functionally
the same position in superantigens I and II so that
substitution will lead to a chimeric form that is able to
function as a superantigen.

The terminology grafted/grafting/graft is used in connection
25 with parts of the full sequence of superantigen II that have
replaced corresponding parts of superantigen I, even if only
one single amino acid has been replaced.

Modified/chimeric superantigens also encompass functional
superantigens modified in other ways, for instance modified by
30 amino acid replacements in regions other than those related to
the invention, conjugated to a target-seeking moiety including
also fused forms when the moiety is a polypeptide/protein. The
order for carrying out the modifications may vary. See below.

SUPERANTIGENS

According to the very first definition (around 1988-1993),
superantigens are bacterial or viral proteins capable of
binding to MHC class II antigens without prior intracellular
5 processing and activate T cells by binding to the β-chain
variable region (Vβ) of the T cell receptor (TCR). The binding
leads to a Vβ family restricted activation of a relatively
large proportion/subset of T cells and lysis of MHC Class II
expressing cells (superantigen dependent cell-mediated
10 cytolysis = SDCC).

Well known wild-type superantigens according to the
definition above are the staphylococcal enterotoxins (SEA, SEB,
SEC1, SEC2, SED, SEE and SEH). Further examples are Toxic Shock
Syndrome Toxin 1 (TSST-1, also of staphylococcal origin),
15 Exfoliating Toxins (EXft), Streptococcal Pyrogenic Exotoxin A,
B and C (SPE A, B and C), Mouse Mammary Tumor Virus proteins
(MMTV), Streptococcal M proteins, Clostridial Perfringens
Enterotoxin (CPET), mycoplasma arthritis superantigens etc. For
a review of superantigens and their properties see Kotzin et al
20 1993.

During the early nineties it was discovered that activation
and subsequent cell lysis could occur in a MHC class II
independent manner in case the superantigen was conjugated with
a target-seeking moiety capable of binding to a cell surface
25 structure (Dohlsten et al WO9201470 and Abrahmsén et al
WO9601650). Upon incubation of target cells (carrying the
target structure for the target-seeking moiety) and effector
cells (T cells) with the conjugates, the target cells become
lysed (superantigen antibody dependent cell-mediated cytolysis
30 = SADCC) without any requirement for class II expression.
Accordingly the superantigen concept of today and used in the
context of the present invention, if not otherwise specified,
encompasses any compound (preferably of polypeptide structure)
that is capable of binding to a cell surface structure (target
35 structure) and to one or more polymorphic TCR chain, in
particular the Vβ chain, thereby activating a subset of T cells

expressing the specific TCR chain involved in the binding. The
T cells then become cytotoxic for cells carrying the surface
structure (target structure, target cells). Normally the
activated subset of T cells constitutes about 1-20% of the
5 total amount of T cells of an individual.

BACKGROUND ART - STRUCTURAL AND FUNCTIONAL STUDIES UTILIZING MUTATED AND
CHIMERIC SUPERANTIGENS.

Chimeric superantigens including point mutated forms have
10 previously been described (Kappler et al WO 9314364, Kappler et
al 1992; Grossman et al 1991; Hufnagle et al 1991; Hartwig et
al 1993; Fraser et al 1993; Mollick et al 1993; Irwin et al
1992; Hudson et al 1993; and Blanco et al 1990). Mollick et al
and Hudson et al show from studies of chimeras that the Vβ
15 specificity of SEA and SEE resides in certain amino acid
sequences present in the carboxy terminal region (i.e. amino
acid residues 200, 206 and 207). In addition to the Vβ
specificity, mainly depending on this region, Mollik et al also
were able to show that for complete reconstitution of SEE like
20 activity of SEA containing SEE grafts towards Vβ8, a fragment
containing the N-terminal 70 amino acid residues from SEE was
needed. This fragment contains parts of the SEE-like MHC class
II α chain binding site and chimeric SEA/SEE molecules
containing this part from SEE, inhibited binding of SEA to MHC
25 class II DR1 in a SEE-like manner.

Recently SEE-SEA chimers involving an exchange of regions
involved in binding to TCRVβ have been described (Lamphaer et
al., J. Immunol. 156 (March 15, 1996) 2178-2185). A SEE
superantigen Fab antibody fusion protein in which the SEE
30 domains involved in the interaction with T cells have been
replaced with the corresponding non-homologous SEA domains has
been discussed at ABRF'96: Biomolecular Techniques, Holiday Inn
Golden Gateway, San Francisco, Califonia March 30 - April 2,
1996 (Björk et al., M45).

35

BACKGROUND ART - THERAPEUTIC USE OF SUPERANTIGENS

Non-conjugated superantigens have been suggested for therapy with curative effect presumably being accomplished through a general activation of the immune system (Kalland et al
5 WO9104053; Terman et al WO9110680 and WO9324136; Newall et al 1991).

It has also been suggested to use modified superantigens conjugated to target-seeking moieties (Dohlsten et al WO9201470; Abrahmsén et al WO9601650, both hereby being
10 incorporated by reference). This enabled a broader therapeutic use of T cell activation through Vβ. The conjugates studied so far have had a diminished class II affinity, which in turn has lead to a decrease of the severe systemic toxicity normally associated with the wild-type superantigens.

15   Terman et al (WO9110680; WO9324136) in side-sentences suggested cancer therapy with modified superantigens and superantigen fragments.

Kappler et al (WO9314634) have suggested to use non-conjugated superantigens (SEB) mutated to have lost their Vβ-
20 binding or MHC Class II binding ability (in the context of vaccines and to neutralize toxic effects of superantigens). Abrahmsén et al (WO9601650) have suggested cancer therapy with conjugated superantigens having a modified, preferably decreased, ability to bind to Class II antigens. The
25 modifications encompassed single mutations as well as construction of chimeras between different superantigens.

THE PROBLEMS THAT HAVE BEEN THE OBJECTIVE TO SOLVE WITH THE PRESENT INVENTION.

The sera of human populations normally contain high titers of
30 antibodies against superantigens. For the staphylococcal superantigens, for instance, the relative titers are TSST-1 > SEB > SEC1 > SE3 > SEC2 > SEA > SED > SEE. These relative titers indicate immunogenicity problems and problems with neutralizing antibodies in case SEs are administered
35 parenterally. Based solely on these problems, SEE should be the preferred staphylococcal superantigen. In the context of work

with fusion proteins, however, we have found that the ability
for T cell MHC class II independent cytotoxicity, superantigen-
antibody dependent cell cytotoxicity (SADCC), of SEE conjugates
is poor. The anti-SE titers also indicate that there might be
5 advantages in modifying a "high titer" superantigen to be more
like a "low titer" superantigen.

THE OBJECTIVES OF THE PRESENT INVENTION.

A first objective is to improve the previously known
10 superantigens with respect to lowering their immunogenicity and
reaction with neutralizing antibodies.

A second objective is to provide superantigens with less side
effects when used as a drug.

A third objective is to provide improved superantigens that
15 can be used as the active principle in the treatment of mammals
suffering from cancers, autoimmune diseases, parasitic
infestations, viral infections or other diseases associated
with cells that on their surface express MHC class II antigens
and/or structures that are specific for respective disease and
20 bind to a target-seeking moiety incorporated into the
superantigen.

THE DISCOVERY THAT HAS RESULTED IN THE INVENTION

A sequence homology analyzis of SEA and SEE (Fig 2) reveals
25 that the non-identical amino acid residues are concentrated to
eight distinct regions. Outside these eight regions, making up
to 34% of the sequence, the identity of the two SEs is 97%,
with conserved amino acid substitutions accounting for the
remaining differences. Four of these regions are structurally
30 close to the two MHC class II binding sites (B: AA 37-50, D:
71-78, E: 136-149, and G 189-195), and are not likely to
interact with the TCR. The additional four regions (A: AA 20-
27, C: 60-62, F: 161-176 and H:200-207) are located on the edge
of the molecule, in the vicinity of the putative TCR binding
35 site, postulated to reside in the groove between the two
subdomains. By grafting the individual regions (replacement of

amino acid residues that differ), we have now found that the
property of SEA-conjugates to induce a cytoxic response as well
as potentiating proliferative response in the absence of MHC
class II, resides in one region in the TCR binding domain of
5 SEA. This Region (A) is transferable to SEE and has a great
impact on activity in the absence of Class II, although limited
effects on the Vβ specificity of the superantigen (Fig 6,
Tab.2). All of the regions (A, C, F and H) seem to participate,
directly or indirectly, in the interaction with the TCR
10 manifested by an altered stimulatory effect on murine T-cell
hybridomas (Tab. 2)

      Due to the analogous mode of action it is conceivable that a
similar structural separation of these TCRVβ binding properties
is at hand also for superantigens analogous to SEA and SEE. The
15 same may also apply within other types of superantigens, in
which the binding structures are organised differently. Our
discovery has enabled us to outline the construction of
chimeric superantigens that potentially are of extremely great
value as therapeutic agents.
20

THE INVENTION

      The first aspect of the invention is a method for the
treatment of a disease in a mammal by activation of its immune
system through administration of a therapeutically effective
25 (immune activating) amount of a modified, preferably chimeric,
superantigen. The mammal is preferably a human. The diseases in
question are mostly associated with cells expressing on their
surface a target structure binding to the superantigen. The
target structure is in most cases different from the TCR
30 epitope normally binding to superantigens. Binding to the
target structure permits also binding to TCR and T cell
activation. Illustrative examples are MHC class II antigens and
other cell surface structures that may be expressed on cells
associated with the courses of diseases. Illustrative diseases
35 are malignant tumors including any type of cancers (such as
carcinoma, sarcoma, melanoma, lymphoma etc.), viral infections,

parasitic infestations and autoimmune diseases. The cancer to
be treated may be located to the colon, breast, cervix, kidney,
stomach, small intestines, duodenum, prostate, testis, skin,
lung, liver, pancreas, skeleton etc including also metastasis
5 at various locations. The inventive active agent is also
applicable to so called multi-drug resistant forms of cancers.
The cells expressing the target structure may also be cells
that in some way control or regulate the development of the
disease to be treated.

10 The characteristic feature of the method is that one employs
a modified superantigen in which one or more amino acid
residues in a region (region I) providing for binding to a
subset of T cells via a polymorphic TCR chain, in particular
TCRVβ, in a wild-type superantigen (SA I) has been replaced
15 with a respective amino acid residue retaining superantigen
activity to the so modified superantigen. The presently
preferred embodiments refer to a chimeric superantigen in which
one or more amino acid residues in a region (region I) of a
first wild-type superantigen (SA I) have been replaced with the
20 corresponding one or more amino acid residues in a
corresponding region (region II) of a second wild-type
superantigen (SA II). The regions I and II differ with respect
to amino acid sequences. The superantigens I and II have been
selected so that the regions I and II can replace each other
25 without killing the superantigen function. In this context one
has to account for the fact that a certain region I alone may
not be interchangeable with the corresponding region of another
wild-type superantigen although when interchanged together with
other regions determining TCR binding and T cell activation,
30 the result becomes a functional active superantigen. The
regions concerned normally comprise less than 20 residues, in
particular for superantigens analogous to SEA. The replacing
amino acid residue thus is different from the replaced residue,
and conceivably includes also conserved substitutions and other
35 amino acid substitutions leading to funtionally active modified
superantigens allowing binding to TCRVβ and activation of a

subset of T cells. This means that the inventively modified
superantigens in its broadest sense encompass any modified
superantigen in which one or more amino acids in the
aforementioned regions have been functionally replaced.

5   The term "conserved substitution" refers to replacement of an
amino acid residue by a chemically similar residue, e.g. a
hydrophobic residue for a different hydrophobic residue, a
charged residue for a different but similarly charged residue
etc.

10   As superantigens I, II etc, the staphylococcal enterotoxins,
in particular those that coordinate zinc, were at the priority
date preferred, i.e. SEA, SEE, SED and possibly also SEH.

The regions involved may have either of the above-mentioned
functions (see the heading "The Discovery that has resulted in
15 the Invention" and the Experimental Part):

1. A great impact on the superantigen activity as such and
   a limited effect on the TCR specificity, in particular
   on Vβ specificity. For SEA-type superantigens this means
   region A (amino acid positions 20-27).

20   2. A profound effect on the specificity with respect to
   binding to polymorphic TCR chains, such as the Vβ chain.
   For SEA-type of superantigens this means regions C
   (amino acid positions 60-62), F (amino acid positions
   110-126) and H (amino acid positions 200-207).

25   For SEA-like superantigens this means one or more of the
substitutions (applied to grafting from SEA to SEE; SEE/A
chimeras):

   Region A: R20G, N21T, S24G, R27K
   Region C: G60D, P62S
30   Region F: H111R, H114Q, G115Y, F117Y, G118N, S124V, G126D
   Region H: D200G, P206S, D207N

At the priority date it was preferred to carry out all
substitutions for each region. For other superantigens,
analogous substitutions between corresponding positions/regions
35 could conceivably also be carried out.

Typically one could start from one first superantigen, like
SEE and SED, and then replace one or more of its unique Vβ
binding regions with the corresponding region(s) of a second
superantigen (e.g. SEA), the first and second superantigens
5 preferably being selected so that the antibody titer in normal
human sera for the first superantigen is lower than for the
second superantigen. For SEA and SEE chimeras, the best modes
correspond to the chimeras SEE/A-A, SEE/A-AH, and SEA/E-BDEG,
with absolute preference for SEE/A-A. See the experimental part
10 and the figures.

Together with the regions A, C, F and H also amino acid
residues at other parts can be exchanged. One type of exchange
is to reduce the class II binding ability, because this
property is associated with common side effects encountered in
15 superantigen therapy (general immune activation with
concomitant systemic release of tumor necrosis factor (TNF) and
interferon-γ). For superantigens such as SEA, SED and SEE,
positions that are important for the ability to coordinate zinc
ions may preferably be changed, i.e. positions 225 and 227, for
20 instance in SEA mutation H225A and in particular D227A will
have a positive impact on reducing toxic side effects (see
Abrahmsén et al WO9601650 and Fraser et al 1993).

Other substitution may be performed althroughout the molecule
as long as they do not destroy the superantigen function, for
25 instance conserved substitutions, in particular outside
regions involved in the binding to class II and TCR. A change
in the DNA sequence for altering the MHC class II binding or
any other change on the DNA level may be carried out either
before or after the change in regions providing for binding to
30 TCR. These other types of modifications can equally well have
been introduced prior to the amino acid replacement in Region
I. In the context of the present invention, the concept of
using a "wild-type superantigen" at the start of the
modification according to the claims thus primarily refers to
35 the wild-type amino acid sequence in region I outside of which
prior modifications may have taken place.

Construction of chimeric and mutated superantigens can be
carried out according to techniques well-known in the art. The
switch from a region specific for one superantigen to the
corresponding region in another superantigen is done on the
5 genomic level and may be accomplished by replacing a complete
sequence or by point mutations of those specific bases that are
required to end up in the desired amino acid sequence. See for
instance the experimental part and also the prior art
references cited above. The term "mutation" comprises
10 replacing, inserting or removing one or more amino acid
residues by modifying the DNA sequence coding for the protein
to be mutated.
   The superantigen to be used in the inventive method can be a
non-conjugated superantigen modified as described above, i.e. a
15 modified superantigen lacking a specifically attached target-
seeking moiety but with a pronounced ability to bind to both
MHC class II antigens and a subset of T cells via TCR. More
preferably the modified superantigen, preferably a chimeric
superantigen, is conjugated to a target-seeking moiety. In the
20 latter case the preferred variants are fusions between the
target-seeking moiety and the modified superantigen. The
conjugates as such are novel and are a separate aspect of the
invention.

25   The structures of the inventive conjugates are analogous to
earlier known antibody-superantigen conjugates (Dohlsten et al
WO9201470; Abrahmsén et al WO9601650, both publications hereby
being incorporated by reference), i.e. the conjugates often
comply with the formula:
30   T-B-SA(m)
where T represents the target-seeking moiety, SA(m) the
modified, preferably chimeric, superantigen as defined above,
and B is a covalent bridge linking T and SA(m) together. T may
in principle contain further superantigen moieties (SA(m)), and
35 SA(m) further target-seeking moieties, although in the

preferred conjugates there is only one target-seeking moiety
and one modified superantigen moiety as defined above.

T can in principle be any structure that is able to bind to a
cell surface structure, preferably a disease specific

5   structure. The structure against which T is directed is usually
different from (a) the Vβ chain epitope to which SA(m) binds,
and (b) the MHC class II epitopes to which superantigens bind.
The target-seeking moiety is primarily selected among
interleukins (e.g. interleukin-2), hormones, antibodies

10 including antigen binding fragments of antibodies, growth
factors etc. See for instance Woodworth, Preclinical and
Clinical development of Cytokine toxins presented at the
conference "Molecular approaches to cancer Immunotherapy",
Ashville, North Carolina, November 7-11, 1993.

15   At the priority date, it was preferred that T was an antibody
(full length antibody, Fab, F(ab)$_2$, Fv, single chain antibody
and any other antigen binding antibody fragment), with
particular emphasis for antibody active fragments (such as
Fab), directed towards the so called C242 epitope (Lindholm et

20 al WO9301303) or more preferably towards the binding epitope
for the lung cancer specific 5T4 antibody (Stern et al
WO8907947). This, however, does not exclude that other cancer
specific antibodies may function equally well or even better.
The term "antibody" comprises monoclonal as well as polyclonal

25 variants, with preference for monoclonal preparations.

T may also be directed towards unique structures on more or
less healthy cells that regulate or control the development of
a disease.

30   The bridge B may be selected as previously described
(Dohlsten et al WO9201470; and Abrahmsén et al WO9601650), i.e.
B shall preferably be hydrophilic and exhibit one or more
structure(s) selected among amide, thioether, disulphide etc.
The most prominent bridges are those obtained by recombinant

35 techniques, i.e. the conjugation takes place at the genomic
level. In such cases oligopeptide bridges containing

hydrophilic amino acid residues, such as Gln, Ser, Gly, Glu, Pro, His and Arg are preferred. Particularly preferred Bs are peptide bridges consisting of 1-10 amino acid residues, with absolute preferences for 3-7 amino acid residues. A typical
5 bridge is the tripeptide GlyGlyPro, SEQ ID NO 1.

The manufacture of the novel inventive conjugates may be carried out in principle according to two main routes: 1. Recombinant techniques and 2. Chemical linking of a target-
10 seeking moiety T to a modified, preferably chimeric, superantigen (SA(m)) as defined above. These methods are well recognized for the ordinary skilled worker and comprise a large number of variants.
Chemical linking of a modified non-conjugated superantigen
15 to a target-seeking moiety T often utilizes functional groups (e.g. primary amino groups or carboxy groups) that are present in many positions in the compounds. It follows that the final product will contain a mixture of conjugate molecules differing in linking positions, as well as hetero- and homo-conjugates.
20 For recombinant conjugates (fusion proteins) the obtained conjugate substance will be uniform with respect to the linking position. Either the amino terminal of the chimeric superantigen is linked to the carboxy terminal of the target-seeking moiety or vice versa. For antibodies, such as intact
25 antibodies and antigen-binding fragments (Fab, Fv, single chain antibodies etc), either the light or the heavy chain may be utilized for fusion. At present time recombinant conjugates are preferred, with utmost preference for Fab fragments and linking of the amino terminal of the chimeric superantigen to the first
30 constant domain of the heavy antibody chain (CH1), without exclusion of the analogous linking to the light chain or to the VH and VL domain that also may give quite good results.
The main host cell for large scale recombinant production of the inventive modified superantigens (fused forms as well as
35 non-conjugated forms) is E. coli. This host provides for in principle two routes: intracellular production and secretion.

The latter variant is preferred because it offers purification of correctly folded proteins from the periplasma and from the culture medium. The above does not exclude that it is possible to produce active conjugates also in other host cells, e.g.
5 eukaryotic cells, such as yeast or mammalian cells.

PHARMACEUTICAL COMPOSITIONS, DOSAGE AND ROUTES OF ADMINISTRATION.
    A third aspect of the instant invention is pharmaceutical compositions containing the inventive modified, preferably
10 chimeric, superantigens as defined above (both conjugated and non-conjugated forms). The compositions contemplated are known in the field, except that now they contain the instant inventive superantigen. Thus, the compositions may be in the form of a lyophilized particulate material, a sterile or
15 aseptically produced solution, a tablet, an ampoule etc. Vehicles such as water (preferably buffered to a physiologically acceptable pH value by for instance PBS) or other inert solid or liquid material may be present. In general terms the compositions are prepared by the conjugate being
20 mixed with, dissolved in, bound to, or otherwise combined with one or more water-soluble or water-insoluble aqueous or non-aqueous vehicles, if necessary together with suitable additives and adjuvants. It is imperative that the vehicles and conditions must not adversely affect the activity of the
25 modified superantigen.
    Normally the inventive superantigen will be sold and administered in predispensed dosages, each one containing an effective amount of the conjugate that, based on the result now presented, is believed to be within the range of 10ng - 50 mg,
30 such as within 10 ng - 1 mg or within 10 µg - 50 mg. The exact dosage will vary from case to case depending on the patient's weight and age, route of administration, type of disease, target-seeking moiety, superantigen, linkage (-B-) etc.
    The administration routes will be those commonly contemplated
35 within the field, i.e. a target cell killing effective amount or therapeutically active amount of a superantigen modified

according to the invention is brought into contact with the
target cells. For the indications specified above this mostly
means parenteral administration, such as injection or infusion
(subcutaneously, intravenously, intraarterial, intramuscularly,
5 intraperitoneal) to a mammal, such as a human being. The
modified, preferably chimeric, superantigens contemplated may
be administered locally or systemically.

By the term "target killing effective amount" is contemplated
that the amount is effective in activating and directing T
10 cells to destroy target cells.

The preferred administration route at the priority date is
the same as contemplated for the superantigen conjugates
according to Dohlsten et al WO9201470 and Abrahmsén et al
WO9601650. This means 1-5 hours' intravenous infusion
15 (preferably 4 hours) per day combined with a fever-reducing
agent (paracetamol). The administration is to be repeated
during some days, for instance 4 days, with care consideration
taken for the risk of boostering antibodies directed towards
the conjugate.
20 The inventive superantigens may be administered either as the
main therapy or in preferred modes as adjuvant therapy in
connection with surgery or other drugs.

In the context of therapy we have found that antibody
25 preparations that are pure with respect to non-covalently
associated heavy and light antibody chains provide advantages
over preparations that contains antibodies in which the chains
are linked together via cystine linkages. Accordingly a fourth
aspect of the invention is the therapeutic use of an antibody
30 preparation, in particular an Fab preparation, in which the
cysteine residues linking the chains together have been
replaced by an amino acid not permitting disulfide formation,
for instance serine. The most preferred antibody specificities
for this aspect of the invention were at the priority date the
35 C242 mab (Lindholm et al., WO9301302) and the 5T4 mab as
defined in the references cited above. In the preferred

variants one of the antibody chains is fused to a superantigen
that is capable of activating a subset of T cells in a Vβ
specific manner as described above. The superantigen may be a
wild-type, a chimera, or a point-mutated version (and
5 combination thereof) as described above or by Dohlsten et al
WO9201470 or by Abrahmsén et al WO9601650. This aspect of the
invention also comprises pharmaceutical compositions as
described above, but containing an antibody preparation as
defined for this aspect of the invention instead of a chimeric
10 superantigen.

   At the priority date it was preferred to use the Fab fragment
5T4 antibody (Stern et al, WO8907947) in combination with the
SEE/A-A chimer with the mutation D227A. The preferred Fab
15 fragment was mutated in both chains in the position providing
interchain disulfide linkage (cys to ser). In order to increase
the yield of the antibody/fusion protein when produced in E
coli, mutations were also carried out in the Vkappa chain at
certain positions. See the experimental part.
20

MATERIALS AND METHODS
Construction of SEA/SEE chimeric genes
   Construction of SEA/SEE chimeras were made using the
polymerase chain reaction (PCR) based method, sequence overlap
25 extension (Horton et al). PCR reactions were performed with
UlTma (Perkin-Elmer) according to manufactures recommendations.
PCR produced fragments were cloned in PCR-script (Stratagene,
USA) and sequenced to verify the correct sequence. The chimeric
superantigen genes were then subcloned in the expression vector
30 pKP889 (Abrahmsén et al 1995), fusing the SE constructs to the
heavy chain portion to the Fab fragment of the murine
monoclonal antibody C215. The SEA and SEE recombinant fusion
proteins were produced as full length polypeptides in
accordance with the consensus sequence for signal peptide
35 cleavage (von Heijne 1986)

Protein expression and purification

The *Escherichia coli* K12 strain UL635 was used for expression
of the Fab-SE fusion proteins and the SEA mutants as described
earlier (Abrahmsén et al 1995). Fab-SE fusion proteins were
5 harvested by centrifugation at 5000 g and the supernatant
fractions were subjected to purification on protein G Sepharose
(Pharmacia Biotech AB, Uppsala, Sweden) as earlier described
(Abrahmsén et al 1995). The purity of the affinity purified
Fab-SE variants were >90% pure when analyzed by SDS-PAGE.

10

Cells

The human B-cell lymphoma cell line Raji and human colon
carcinoma Colo 205 were cultured in complete R-medium (RPMI-
1640 supplemented with 10% fetal calf serum (Gibco BRL, Life
15 Technologies, Ltd. Paisley Scotland) 1 mM glutamine; HyClone
Europe, Ltd. Cramlington, $5x10^{-5}$ M β-mercaptoethanol; ICN
Biomedicals INC. Costa Mesa CA, 0.1 M NaHCO3; Seromed
Biochrome, $1x10^{-2}$ M Hepes buffer; HyClone Europe, Ltd.
Cramlington., 0,1 mg/ml gentamycine; Biological Industries
20 Kibbutz Beit Haemek Israel, $1x10^{-3}$ M sodium pyruvate; HyClone
Europe, Ltd. Cramlington). CHO cells transfected with human
C215 and CD80 molecules were cultivated in complete R-medium
supplemented with 0.5 mg/ml Geniticin (G418) Gibco BRL, Life
Technologies, Ltd. Paisly Scotland). Peripheral blood
25 mononuclear cells (PBM) were prepared from heparinized blood
from normal donors. The cells were isolated by density
centrifugation over Ficoll-Paque as previously described
(Dohlsten et al 1991). Human T lymphocytes were purified to
homogenicity by positive selection using MiniMACS columns in
30 conjunction with magnetic beads coated with monoclonal
antibodies specific for human CD4 and CD8 (Miltenyi Biotec
GmbH, Germany) according to the manufacturers specifications.
Human SEA and SEE reactive cell lines were generated as
previously described (Dohlsten et al 1994). Human TCR Vβ22
35 expressing cell line was generated from a primary stimulated
SEA reactive cell line using positive selection with magnetic

Dynabeads (Dynal A.S., Norway) coated with TCR Vβ22 specific monoclonal antibody (Immunotech, France). Enriched cells contained > 95 % TCR Vβ22^{+} T cells as determined by flow cytometry (data not shown). Murine T-cell hybridomas (I1B3, 2B4

5 and 11.40) were generated as described (Fleury et al 1991).

Cytotoxicity assay

Cytotoxicity was measured in a standard ^{51}Cr release assay after 4 or 6 hours as previously described (Dohlsten et al

10 1991). Human Colo205 or Raji cells were used as target cells. The effector cells, either SEA or SEE reactive human T cell lines or TCR Vβ22 cell lines, were added at an effector to target ratio of 30:1. ^{51}Cr-labeled target cells were used in the cytotoxicity assays at 2500 cells/200 ml complete medium in

15 V-bottomed microtiter wells. C215Fab-SEA/E hybrids were added at various concentrations as indicated and ^{51}Cr release was measured in a g-counter. The percentage specific cytotoxicity was calculated as 100x[(c.p.m. experimental release - c.p.m. background release)/(c.p.m. total release - c.p.m. background

20 release)].

Lymphocyte proliferation assays

To measure proliferation 10^5 human T cell responders were incubated at 37°C with 10^4 irradiated (20.000 Rad) stimulator

25 cells in 200 ml complete medium in U-shaped 96-well microtitre plates with varying amounts of C215Fab-SEA/E hybrids for 72 hours. Proliferation was estimated by incorporation of [^{3}H]-thymidine as described (Dohlsten et al 1988).

30 **Analysis of Fab-SAg induced IL-2 production.**

Murine T-T hybridoma cells (10^5) were incubated in 200 ml complete R-medium with C215Fab-SEA/E chimeric proteins in the presence of $2x10^4$ Raji stimulator cells. After 48 hours, supernatants were harvested and analyzed for presence of murine

35 IL-2. Briefly, cytokine content was analyzed using rat anti-mouse cytokine mAb as catcher antibodies. Purified rat anti-

mouse IL-2, biotin-labeled rat anti-mouse IL-2, rIL-2 was purchased from PharMingen (San Diego, CA). Biotin-labeled anti-cytokine mAb, Vectastain ABC kit (Vector Laboratories, CA) and peroxidase substate kit (Bio-Rad Laboratories, CA) were used
5 for detection of cytokines. The absorbance was determined in a ImmunoReader NJ2000 (InterMed Roskilde, Denmark) at 405 or 450 nm.

Mutation of 5T4 Fab

10 *Construction of a vector for expression of 5T4Fab-SEA in E. coli.*

The Fv-encoding portions of 5T4 were cloned from the 5T4 hybridoma, obtained from Dr Peter Stern (Stern et al., WO8907947). In more detail: cDNA was made from the mRNA,
15 regions of the entire variable domains and parts of the signal sequences as well as the first constant domain of the heavy chain and the constant domain of the light chain were amplified by PCR. The oligonucleotides

5'-CAATTTTCTTGTCCACCTTGGTGC-3' (SEQ ID NO: 2) and
20 5'-ACTAGTCGACATGGATGGAGCTITATCATIyTCTT-3' (SEQ ID NO 3)
were used for the heavy chain, resulting in a 553 bp fragment, while the oligonucleotides

5'-ACTAGTCGACATGGGCITCAAGATGGAGTCACAkwyyCwGG-3' (SEQ ID NO: 4) and
25 5'-GCGCCGTCTAGAATTAACACTCATTCCTGTTGAA-3' (SEQ ID NO: 5)
were used for the light chain, yielding a 724 bp fragment. For each chain three separate clones were sequenced and found to be identical. DNA fragments suitable for insertion into the expression vector (ref) were obtained in a second PCR step. In
30 order to assemble a Fab-expression plasmid, the variable regions of 5T4 were fused to sequences coding for constant regions from the murine IgG1/k antibody C242 mab (Lindholm et al, WO9301302). A region coding for a superantigen derived from staphylococcal enterotoxin A (SEA) was fused after the heavy
35 chain. The verified sequence for the Vkappa chain antibody framework for the 5T4 antibody is given in the results.

Mutagenesis of 5T4

Seven amino acid replacements were introduced in the regions coding for the antibody framework. These were Phe10Ser,
5 Thr45Lys, Ile63Ser, Tyr67Ser, Phe73Leu, Thr77Ser and Leu78Val. Similarly, the Cys residues in either chain involved in the interdomain disulfide bond were replaced by serine residues resulting in the mutations Cys458Ser in the heavy chain and Cys214Ser in the light chain. The mutations were introduced
10 using PCR-based mutagenesis and the DNA sequence obtained was confirmed using sequencing.

Fermentor expression and purification of 5T4Fab-SEA.

The expression plasmid contains the kanamycin resistance gene
15 and a lacUV5-promoter that may be induced with IPTG. The fusion proteins were purified from the clarified culture medium using protein G Sepharose and SP-Sepharose (Pharmacia Biotec, Uppsala, Sweden) and formulated in citrate buffer using Sephadex G-25, essentially as described. Characterization using
20 SDS-PAGE, reverse phase HPLC and mass spectrometry showed that the purified fusion protein was more than 95 % pure and had the correct molecular mass.

RESULTS: SUPERANTIGEN MODIFICATIONS.

25   The superantigen dependent cellular cytotoxicity (SDCC) of C215Fab-SEA and of C215Fab-SEE against MHC class II$^+$ Raji cells, was analyzed using SEA- and SEE-reactive human T cells as effector cell lines. Despite the difference in Vβ specificity between SEA and SEE both superantigens exhibited
30 induction of comparable degree of cytotoxicity with both effector cell lines (Fig. 1). To discriminate between effects of MHC class II presentation and direct effects of SEA and SEE in TCR recognition, they were examined in superantigen-antibody dependent cellular cytotoxicity (SADCC) against C215 expressing
35 Colo205 cells. In this assay the Fab moiety directs the fusion protein to C215-expressing target cells and results in the

presentation of fused SE molecules to cytotoxic T-cells (CTL)
independent of MHC class II molecules (Dohlsten et al 1994).
Despite >80% amino acid sequence identity between SEA and SEE
the TCR interaction of SEA and SEE displays qualitative

5 differences in this type of assay. The C215Fab-SEA fusion
protein retains its ability to direct SEA and SEE reactive CTL
against the MHC class II⁻ target cells (FIG 1) while C215Fab-
SEE fails to induce cytotoxicity of the, MHC class II⁻ target
cells, neither with SEA nor with SEE reactive CTL (FIG 1).

10 It has previously been reported by other investigators that
the differences in Vβ specificity between SEA and SEE primarily
relates to a three amino acid difference in the loop preceding
and in the irregular a5 helix (Irwin et al 1992, Hudson et al
1993, Fraser et al 1993, and Mollick et al 1993). The

15 difference in respect to TCR interaction reported in this
investigation is not related to altered TCR Vβ specificity
since the ability of C215Fab-SEA to induce MHC class II
independent cytotoxicity is not restricted to SEA reactive CTL
but is also seen with SEE reactive CTL.

20 Sequence homology analysis of SEA and SEE (Fig. 2) reveals
that the non-identical amino acid residues are concentrated to
eight distinct regions. Outside these eight regions, making up
to 34% of the sequence, the identity of the two SE's is 97%,
with conserved amino acid substitutions accounting for the

25 remaining differences. Four of the non-homologous regions are
structurally close to the two MHC class II binding sites (B, D,
E and G), and are not likely to interact with the TCR (Fig. 3).
The additional four regions (A: AA 20-27, C: 60-62, F: 161-176
and H: 200-207) are located on the edge of the molecule (Fig.

30 3), in the vicinity of the TCR binding site, located in the
groove between the two subdomains (Kappler et al 1992). To
investigate the qualitative difference in TCR recognition
between SEA and SEE we made hybrid proteins by grafting the
regions from SEA to SEE as single region chimeras (SEE/A-A, -C,

35 -F, H) as double region hybrids (SEE/A-AH) and by grafting the
regions located in the vicinity of the MHC class II binding

sites on SEE to SEA (SEA/E-BDEG) (Fig 4). All of the chimeric
SEs were expressed as C215Fab fusion proteins to be able to
detect differences with respect to their activity in the
absence of MHC class II.

5

**The SEA/E hybrid proteins in fusion with the C215Fab moiety
displays difference in Fab targeted cytotoxic assays.**

The SDCC activity of C215Fab-SEE/A hybrid proteins against
MHC class II+ Raji cells were analyzed using SEA-reactive human
10 T cells as effectors. The EC_{50} values of all C215Fab-SE hybrids
as well as the C215Fab-SEAwt and -SEEwt falls in the margin of
errors (e.g. 10^{-12}-10^{-11} M, Fig 5). The only detectable
difference is a slightly reduced plateau for the C215Fab-SEE/A-
AH hybrid, indicating a loss of responding T cells. On the
15 other hand in SADCC experiments where the cytotoxicity is
directed towards MHC class II-/C215+ Colo 205 cell line, only
C215Fab-SEE/A-A, C215Fab-SEE/A-AH and C215Fab-SEA/E-BDEG
induced comparable cytotoxicity as the C215Fab-SEAwt (Fig 5).
The C215Fab-SEE/A-F hybrid is able to induce C215 targeted
20 cytotoxicity at higher concentrations (EC_{50} >10^{-10} M). Although
the C215Fab-SEE/A-H hybrid is able to induce C215 targeted
cytotoxicity with similar half maximal concentration as
C215Fab-SEAwt (e.g. EC_{50} 10^{-13} M), the absolute level of
cytotoxicity is strongly reduced (Fig 5). This difference could
25 be a consequence of a restricted Vβ specificity of the C215Fab-
SEE/A-H while the ability of inducing C215 targeted
cytotoxicity prevails in the responding T cell sub-population.
To further investigate this notion we prepared human Vβ22
oligoclonal CTL line. Human Vβ22 are analogous to murine Vβ3 in
30 the respect that it is a SEA non SEE specific Vβ family. It has
previously been shown (Mollick et al 1993) that the major
contribution of SEA and SEE Vβ is primarily residing in the
three amino acid difference between SEA and SEE in region H (AA
200-207). In SDCC assays against MHC class II+ Raji targets,
35 using the Vβ22 oligoclonal CTL line as effectors, only hybrids
containing the SEA-H region are able to give C215Fab-SEAwt-like

response (e.g. C215Fab-SEE/A-H, C215Fab-SEE/-AH and C215Fab-SEA/E-BDEG, Fig. 6). The C215Fab-SEE/A-A hybrid, that was able to induce a full SDCC response with whole CTL populations as effectors is in this assay strongly reduced both in half
5 maximal concentration and in the plateau (Fig. 6). When the cytotoxicity of the Vβ22 CTL is directed towards the MHC class II$^-$/C215$^+$ Colo 205 cell line only hybrids containing both SEA-A and SEA-H (e.g. C215Fab-SEE/A-AH and C215Fab-SEA/E-BDEG) regions are able to induce a cytotoxic response, comparable to
10 a C215Fab-SEAwt (Fig. 6). The hybrid containing only the SEA region A (C215Fab-SEE/A-A) induces a lower level of cytotoxicity with a comparable EC50 value. This indicates that the remaining activity seen with the C215Fab-SEE/A-H hybrid in SADCC with the whole T cell population as effectors is not a
15 consequence of the hybrid induced response in restricted population of T cells. A more likely explanation for the observation is that the ability to induce a SADCC response of the C215Fab SE hybrid proteins is primarily residing in the SEA-A region with a minor contribution from the SEA-H and -F
20 regions. There is no evidence that this quality is restricted to any subset of T cells in the combined SEA-SEE responding T cell population, since C215Fab SEA is able to induce the same response with as well with SEE reactive CTLs and C215Fab-SEE/A-A is able to fully to reconstitute the response seen with
25 C215Fab-SEA.

The SEA/E hybrid proteins in fusion with the C215Fab moiety displays difference in Fab targeted proliferation assays.

It has been previously shown that purified resting human T
30 cells are induced to proliferate by presentation of C215Fab-SEA on a MHC class II$^-$/C215$^+$/CD80$^+$ cell line (Lando et al 1993). The ability of C215Fab-SEA to induce MHC II independent proliferation is however markedly reduced with C215Fab-SEE (Tab. 1). To investigate if this difference in quality shows
35 the same confinement to SEA region A, as was seen with SADCC, we investigated the proliferative capacity of C215Fab-SE

hybrids, presented by either CHO-DR1+/CD80+ or CHO-C215+/CD80+
transfected cell lines, on purified resting human T cells. When
presenting the Fab-SE conjugates on CHO-DR1+/CD80+ no
differences between the different SE proteins were noted (data
5 not shown). However grafts of SEA region A, C and H in SEE
potentates the proliferative activity compared to C215Fab-SEE.
The best results were obtained by grafting SEA regions A and H,
indicating a important role for region A as was seen for the
MHC class II independent cytotoxicity. By using a negative
10 selection it is possible that the differences between Fab-SEA
and -SEE would be more prominent.

Vβ specificity of SE-hybrids
     To further investigate if the C215Fab-SEA/SEE hybrid-fusion
15 proteins were associated with a certain Vβ specificity we used
SEA reactive murine T cell hybridomas expressing Vβ1, Vβ3 and V
β11. It is obvious from the data obtained that all of the
regions investigated, directly or indirectly, affects the
interaction with the TCR. By grafting SEA regions C and F in
20 C215Fab-SEE the activity towards the SEA and SEE cross reactive
Vβ1 hybridoma I1B3 is destroyed. The same chimeras seems to
have no or minor effects on the activity of Vβ3 and Vβ11
hybridomas (2.B4 and 11.40) in comparison with C215Fab-SEE. By
grafting SEA region A in C215Fab-SEE the activity towards Vβ3
25 (2.B4) is enhanced by at least a factor 100, in comparison to
C215Fab-SEE. More pronounced effects are seen with the same
cell line by grafting SEA region H in C215Fab-SEE. This
pronounced effect on the influence of Vβ3 specificity by SEA
region H has also been noted by earlier investigations (Mollick
30 et al 1993). The same chimera however (C215Fab-SEE/A-H), seems
to reduce the activity towards the SEA/SEE cross reactive Vβ1
and Vβ11 hybridomas (I1B3 and 11.40) by a factor 10. In
conclusion, the TCR interaction of SEA seems to involve all of
the SEA-SEE, variable regions A, C, F and H.
35

Seroreactivity

The seroreactivity in human serum samples towards the chimeric SEs was investigated both in pooled samples from different parts of the world as well as in individual serum
5 samples. By grafting both SEA regions A and H in SEE we obtained an intermediate seroreactivity (Fig 8). A similar seroreactivity was also seen against the chimera C215Fab-SEE/A. However, single grafts of SEA region A in SEE (C215Fab-SEE/A-A) gave a C215Fab-SEE like seroreactivity, indicating that SEA
10 region H is responsible for the remaining seroreactivity against C215Fab-SEE/A-AH. This indicates that the SEA region H is part of dominating antigenic epitope in SEA. The seroreactivity from pooled serum samples from other parts of the world (Japan and USA) as well as 14 individual samples from
15 Sweden all confirms the same general pattern (data not shown).

Results: Mutations of the Fab part of the fusion proteins.
Expression of 5T4FabSEA-constructs

The production level in *E.coli* of 5T4Fab-SEA in the fermenter
20 was found to be significantly lower than other Fab-superantigen constructs previously studied in our lab. Two types of modifications were therefore introduced to increase the production level. Firstly, seven different point mutations in the framework region of the light chain were introduced. These
25 were Phe10Ser, Thr45Lys, Ile63Ser, Tyr67Ser, Phe73Leu, Thr77Ser and Leu78Val. Secondly, the cysteine residues making the disulfide bond connecting the heavy and the light chains were replaced by serine residues. The latter modification resulted in a three-fold increase and the 7 point mutations in an
30 additional 12-fold increase in the production level. In addition to the significantly increased production level, removing the disulfide bond also gives a more homogenuous product since the possibility of the these reactive thiol groups to react with other thiol containing agents is excluded.
35 The modified 5T4 molecule was checked for affinity to its antigen as well as for biological activity in SADCC. No

differences between the mutant form and the wildtype form could be detected in these assays.

The Cys/Ser mutation was also performed in the heavy and light chains of the Fab fragments of several other monoclonal antibodies. The products became homogenous and fully retained the antigen binding capability.

Sequence of region of the antibody frame work for the 5T4 Vkappa chain:

DAVMTQTPTF LLVSAGDRVT ITCKASQSVS NDVAWYQQKP GQSPTLLISY   50
TSSRYAGVPD RFIGSGYGTD FTFTISTLQA EDLAVYFCQQ DYNSPPTFGG   100
GTKLEIK (SEQ ID NO 6)

Underlined sequences are CDRs. Bold-typed positions were mutated: Phe10Ser, Thr45Lys, Ile63Ser, Ile63Thr, Tyr67Ser, Phe73Leu, Thr77Ser, Leu78Val.

	Proliferation EC$_{50}$ (pM)
C215Fab-SEAwt	2.2
C215Fab-SEEwt	6.9
C215Fab-SEE/A-A	0.9
C215Fab-SEE/A-C	2.8
C215Fab-SEE/A-F	5.7
C215Fab-SEE/A-H	1.0
C215Fab-SEE/A-AH	0.3
C215Fab-SEA/E-BDEG	1.6

Table 1.

	11B3 (MuVβ 1) EC$_{50}$ (nM)	2.B4 (MuVβ 3) EC$_{50}$ (nM)	11.40 (MuVβ 11) EC$_{50}$ (nM)
C215Fab-SEA	10	3	0.05
C215Fab-SEE	10	>1000	0.05
C215Fab-SEE/A-A	10	10	0.05
C215Fab-SEE/A-C	>1000	>1000	0.05
C215Fab-SEE/A-F	>300	>300	0.05
C215Fab-SEE/A-H	100	3	0.3
C215Fab-SEE/A-AH	10	3	0.3

Table 2.

Figure legends

Figure 1. MHC class II dependent and independent cytotoxicity with human SEE and SEA CTL.

MHC class II dependent cellular cytotoxicity(A and B) and C215

dependent cellular cytotoxicity (C and D) with C215Fab-SEA (■)

and C215Fab-SEE (♦) as effector molecules. Cytotoxicity was

analyzed in a standard 4-h ⁵¹Cr release assay using a SEE-

reactive human T-cell line (A and C) a SEA-reactive human T-

cell line (B and D). Target cell lines were MHC class II⁺/ C215⁻

Raji (A and B) and MHC class II⁻/ C215⁺ Colo 205 (C and D). Data

are from single assays that is representative of two (A and C)

to five (B and D) independent experiments.

Figure 2. Homology alignment of SEA and SEE.

SEA/SEE variable regions close to the TCR binding site (A, C, F

and H) and variable regions close to the two MHC class II

binding sites.

Figure 3. Cartoon model of SEA.

Molscript model (Kraulis, 1991) of the SEA crystal (Schad et

al. 1995). SEA/SEE variable regions close to the TCR binding

site (A, C, F and H) and variable regions close to the two MHC

class II binding sites. The zinc ion is a round ball.

Figure 4. Schematic representation of chimeric SE molecules.

Stretches of SEA sequence are depressed. SEA/SEE variable

regions are represented by A, B, C, D, E, F, G and H.

**Figure 5. MHC class II dependent and independent cytotoxicity
with human SEA reactive CTL.**

(A) MHC class II dependent cellular cytotoxicity and (B) C215
dependent cellular cytotoxicity of C215Fab-SEE/A-A (♦),C215Fab-

5 SEE/A-C (□), C215Fab-SEE/A-F (◊), C215Fab-SEE/A-H (●), C215Fab-
SEE/A-AH (Δ)and C215Fab-SEA/E-BDEG (O). Cytotoxicity was
analyzed in standard 4-h ^{51}Cr release assay using a SEA-reactive
human T-cell line. Target cell lines were MHC class II⁻/ C215⁻
Raji (A) and MHC class II⁻/ C215⁺ Colo 205 (B). Data are from

10 single assays that is representative of five independent
experiments.

**Figure 6.   MHC class II dependent and independent cytotoxicity
with human Vb22⁺ CTL.**

15 (A) MHC class II dependent cellular cytotoxicity and (B) C215
dependent cellular cytotoxicity C215Fab-SEA (■), C215Fab-SEE
(♦), C215Fab-SEE/A-A (♦),C215Fab-SEE/A-C (□), C215Fab-SEE/A-F
(◊), C215Fab-SEE/A-H (●), C215Fab-SEE/A-AH (Δ)and C215Fab-
SEA/E-BDEG (O) as effector molecules. Cytotoxicity was

20 analyzed in a standard 4h ^{51}Cr release assay using a Vb22
selected SEA-reactive human T-cell line. Target cell lines were
MHC class II⁺/ C215⁻ Raji ((A) and MHC class II⁻/ C215⁺ Colo 205
(B). Data are from single assays that is representative of two
independent experiments.

25

**Figure 7. MHC class II dependent and independent proliferation
(not in the priority applications).**

Effects of Fab-SE hybrids on MHC class II dependent (A) and
independent (B) T cell proliferation. Purified human T-cells

30 were stimulated for 96h with C215Fab-SEA (■), C215Fab-SEE (♦),

C215Fab-SEE/A-A (•),C215Fab-SEE/A-C (□), C215Fab-SEE/A-F (◊),

C215Fab-SEE/A-H (•), C215Fab-SEE/A-AH (Δ)and C215Fab-SEA/E-BDEG

(O) presented on MHC class II⁺/C215⁻ CHO-DR4/C215

transfectants (A) and on MHC class II⁻/C215⁺ CHO-CD80/C215

5 transfectants (B). After 72h the cells were pulsed with [³H]-

thymidine for 24h and incorporated label were measured and

represented as half maximal concentration (EC$_{50}$). Data are from

a single assays that is representative of two independent

experiments.

10

Figure 8. Seroreactivity in a human Ig pool.

Pool of >5000 sera from healthy donors in Southern Europe

against C215Fab-SE fusion proteins. Serially diluted human Ig

was allowed to interact for 1h at room temperature with

15 C215Fab-SEAwt, C215Fab-SEEwt, C215Fab-SEE/A-A, C215Fab-SEE/A-H

and FabSEE/A-AH. Immobilized to the micro titer plates at a

concentration of 1 ng/well. Correction for C215Fab binding to

serum proteins was made by subtracting the OD-value for C215Fab

at each point. Each point represents the mean of duplicate

20 samples. For further details see Materials and Methods.

Table 1. Purified human T-cells were stimulated for 96h with

respective C215Fab-SE presented on MHC class II negative CHO-

CD80/C215 transfectants. After 72h the cells were pulsed with

25 ³H-thymidine for 24h and incorporated label was measured and

represented as half maximal concentration (EC$_{50}$).

Table 2. Murine T cell hybridomas were stimulated for 48h with

native or chimeric Fab conjugated superantigen. Activity was

measured as IL-2 production and represented as half maximal
concentration (EC_{50}).

Work during the priority year.

5   In an attempt to minimize the toxicity of the superantigen
chimer antibody fusion C242Fab-SEE/A-A, the chimer SEE/A-A has
been mutated in Class II binding sites as described in
WO9601650 and fused to C215Fab. The constructions are C215Fab-
SEE/A-A-D227A, C215Fab-SEE/A-A-F47A/D227A, C215Fab-SEE/A-A-
10   H187A/D227A, C215Fab-SEE/A-A-W130A/D227A, C215Fab-SEE/A-A-
D70A/D227A, C215Fab-SEE/A-A-N50S/D227A, C215Fab-SEE/A-A-
N50S/D70A/D227A, C215Fab-SEE/A-A-F47Y/D227A och C215Fab-SEE/A-
A-D70R/D227A. All fusions have been tested for their ability to
induce proliferation of human PBMC in order to identify mutants
15   having a lowered activity compared to C215Fab-SEE/A-A-D227A. A
lowered proliferative activity has been observed for C215Fab-
SEE/A-A-F47A/D227A, C215Fab-SEE/A-A-F47Y/D227A och C215Fab-
SEE/A-A-D70R/D227A. Some of the chimeric fusions have also been
tested for antibody titer in human normal serum (C215Fab-SEE/A-
20   A-D227/A, C215-FabSEE/A-A-D70A/D227A and C215Fab-SEE/A-A-
D70R/D227A). A comparison was made with C215Fab-SEA-D227A and
C215Fab-SEE-D227A. Relative C215FabSEA-D227A, the titer was
much lower for each chimer tested. Relative C215Fab-SEE-D227A,
the titer was slightly higher for each chimer tested.
25   This means replacements at positions corresponding to one or
more, preferably two, of the positions 47, 50, 70, 130, 187,
227 as defined in sequence ID nos 7 and 8 in figure 2.

Because many varying and different embodiments may be made
30   within the scope of the inventive concept herein taught, and

because modifications may be made in the embodiments herein detailed in accordance with the descriptive requirements of the law, it is to be understood that the details herein are to be interpreted as illustrative and not in a limiting sense.

REFERENCES

Abrahmsén L et al (1995) Characterization of two distinct MHC Class II binding sites in the superantigen staphylococcal enterotoxin A. EMBO J 14:2978-86.

Abrahmsén et al (1996) WO9601650 (patent application). A conjugate between a modified superantigen and a target-seeking compound and the use of the conjugate.

Antonsson P et al (1996) Staphylococcal enterotoxin A and E chimera with reduced seroreactivity and retained ability to target cytotoxic T cells for use in tumor therapy. ABRF '96: Biomolecular Techniques, Holiday Inn Golden Gateway, San Francisco, California. March 30-April 2, 1996.

Dohlsten et al (1988) Two subsets of human peripheral blood CD4+ T helper cells differing in the capacity to produce IL-2 and interferon-gamma can be defined by the Leu-18 and UCHL1 monoclonal antibodies. Eur J Immunol 18:1173-1178.

Blanco et al (1990) Mutants of staphylococcal toxic shock syndrome toxin 1: Mitogenicity and recognition by a neutralizing monoclonal antibody. Infect. Immun. 58 (1990) 3020-3028.

Dohlsten M et al (1994) Monoclonal antibody-superantigen fusion proteins: Tumor specific agents for T cell based tumor therapy. Proc Natl Acad Sci USA 91:8945-8949.

Dohlsten M et al (1991) Monoclonal antibody-targeted superantigens: A different class of anti-tumor agents. Proc Natl Acad Sci USA 88:9287-9291.

Dohlsten et al (1992) WO9201470 (patent application). Target specific antibody -superantigen conjugates and their preparation.

Fleury S et al (1991) Mutational analysis of the interaction between CD4 and class II MHC: class II antigens. Cell 66:1037-49.

Fraser JD et al (1993) Structural model of Staphylococcal enterotoxin A interactions with MHC class II antigens. In: Huber,BT Palmer, E (eds) Current Communications in Cell and Molecular Biology 7. Cold Spring Harbour Laboratory Press, Cold Spring Harbor, NY. pp 7-29.

Grossman et al (1991) Mutation of the disulfide loop in staphylococcal enterotoxin A. Consequences for T cell recognition. J Immunol 147:3274-3281.

Hartwig UF et al (1993) Mutations affecting MHC class II binding of the superantigen streptococcal erythrogenic toxin A. Int. Immunol. 5 (8) 869-875.

Horton RM et al (1990) Gene splicing by overlap extension: tailor-made genes using the polymerase chain reaction. Biotechniques 8:528-535

Hudson et al (1993) Two adjacent residues in staphylococcal enterotoxins A and E determine T cell receptor V beta specificity. J Exp Med 177:175-184.

Hufnagle WO et al (1991) The carboxyl-terminal region of staphylococcal enterotoxin type A is required for a fully active molecule. Infect Immun 59:2126-2134.

Irwin MJ et al (1992) Enterotoxin residues determining T-cell receptor Vb binding specificity. Nature 359:841-3

Kalland et al (1991) WO 9104053 (patent application).
Pharmaceutical composition that makes cells expressing MHC
Class II antigens targets for cytotoxic cells.

Kappler JW et al (1992) Mutations defining functional regions
5 of the superantigen staphylococcal enterotoxin B. J Exp Med
175:387-396

Kappler et al (1993) WO9314634 (patent application). Protective
effects of mutated superantigens.

Kotzin BL et al (1993) Superantigens and their potential role
10 in human disease. Adv Immunol 54:99-166.

Kraulis PJ (1991) MOLSCRIPT: A program to produce both detailed
and schematic plots of protein structures. J Appl Cryst 24:946-
950.

Lamphear JG et al (1996) Residues near the amino and carboxy
15 termini of staphylococcal enterotoxin E independently mediate
TCRVβ-specific interactions. J Immunol 156: 2178-2185 (March
15, 1996)

Lando PA et al (1993) Co-stimulation with B7 and targeted
superantigen is required for MHC class II-independent T-cell
20 proliferation but not cytotoxicity. Immunology 80: 236-241.

Lindholm et al (1993) 9301302 (patent application). Tumor,
carbohydrate antigen specific monoclonal antibody and cell
line.

Mollick JA et al (1993) Localization of a site on bacterial
25 superantigens that determines T cell receptor beta chain
specificity. J Exp Med 177:283-293.

Newall et al (1991) In vivo T-cell activation by staphylococcal
enterotoxin B prevents outgrowth of a malignant tumor. Proc
Natl Acad Sci USA 88 1074-1078

Schad EM et al (1995) Crystal structure of the superantigen,
Staphylococcal enterotoxin type A. EMBO J 14:3292-3301

Stern et al (1989) WO8907947 (patent application). Improvements
relating to anigens.

5 Terman et al (1991) WO9110680 (patent application). Tumor
killing effects of enterotoxins and related compounds.

Terman et al (1993) WO9324136 (patent application). Tumor
killing effects of enterotoxins and related compounds.

10 von Heijne, G (1986). A new method for predicting signal
sequence cleavage sites. Nucleic Acid Res, 14, 1483-1490.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 3 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

    Gly Gly Pro
    1

(3) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

    CAATTTTCTT GTCCACCTTG GTGC

(4) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 35 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

    ACTAGTCGAC ATGGATGGAG CTITATCATI YTCTT

(5) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 41 base pairs
             (B) TYPE: nucleic acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

        ACTAGTCGAC ATGGGCITCA AGATGGAGTC ACAKWYYCWG G

(6) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 34 base pairs

             (B) TYPE: nucleic acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        GCGCCGTCTA GAATTAACAC TCATTCCTGT TGAA

(7) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 107 amino acids
             (B) TYPE: amino acid
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp 1	Ala	Val	Met	Thr 5	Gln	Thr	Pro	Thr	Phe 10
Leu	Leu	Val	Ser	Ala 15	Gly	Asp	Arg	Val	Thr 20
Ile	Thr	Cys	Lys	Ala 25	Ser	Gln	Ser	Val	Ser 30
Asn	Asp	Val	Ala	Trp 35	Tyr	Gln	Gln	Lys	Pro 40
Gly	Gln	Ser	Pro	Thr 45	Leu	Leu	Ile	Ser	Tyr 50
Thr	Ser	Ser	Arg	Tyr 55	Ala	Gly	Val	Pro	Asp 60
Arg	Phe	Ile	Gly	Ser 65	Gly	Tyr	Gly	Thr	Asp 70

```
Phe   Thr   Phe   Thr   Ile   Ser   Thr   Leu   Gln   Ala
                        75                            80

Glu   Asp   Leu   Ala   Val   Tyr   Phe   Cys   Gln   Gln
                        85                            90

Asp   Tyr   Asn   Ser   Pro   Pro   Thr   Phe   Gly   Gly
                        95                           100

Gly   Thr   Lys   Leu   Glu   Ile   Lys
                       105
```

(8) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 233 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Ser   Glu   Lys   Ser   Glu   Glu   Ile   Asn   Glu   Lys   Asp   Leu
                        5                             10

Arg   Lys   Lys   Ser   Glu   Leu   Gln   Gly   Thr   Ala   Leu   Gly
                  15                      20

Asn   Leu   Lys   Gln   Ile   Tyr   Tyr   Tyr   Asn   Glu   Lys   Ala
 25                           30                            35

Lys   Thr   Glu   Asn   Lys   Glu   Ser   His   Asp   Gln   Phe   Leu
                   40                       45

Gln   His   Thr   Ile   Leu   Phe   Lys   Gly   Phe   Phe   Thr   Asp
       50                         55                             60
```

| His | Ser | Trp | Tyr | Asn 65 | Asp | Leu | Leu | Val | Asp 70 | Phe | Asp |

Ser Lys Asp Ile Val Asp Lys Tyr Lys Gly Lys Lys
          75   80

Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys
85   90   95

Ala Gly Gly Thr Pro Asn Lys Thr Ala Cys Met Tyr
               100   105

Gly Gly Val Thr Leu His Asp Asn Asn Arg Leu Thr
     110   115   120

Glu Glu Lys Lys Val Pro Ile Asn Leu Trp Leu Asp
               125   130

Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys
          135   140

Thr Asn Lys Lys Asn Val Thr Val Gln Glu Leu Asp
145   150   155

Leu Gln Ala Arg Arg Tyr Leu Gln Glu Lys Tyr Asn
               160   165

Leu Tyr Asn Ser Asp Val Phe Asp Gly Lys Val Gln
     170   175   180

Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro
               185   190

Ser Val Asn Tyr Asp Leu Phe Gly Ala Gln Gly Gln
          195   200

Tyr Ser Asn Thr Leu Leu Arg Ile Tyr Arg Asp Asn
205   210   215

Lys Thr Ile Asn Ser Glu Asn Met His Ile Asp Ile
               220   225

Tyr Leu Tyr Thr Ser
     230

(9) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 233 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ser  Glu  Lys  Ser  Glu  Glu  Ile  Asn  Glu  Lys  Asp  Leu
               5                         10

Arg  Lys  Lys  Ser  Glu  Leu  Gln  Arg  Asn  Ala  Leu  Ser
          15                   20

Asn  Leu  Arg  Gln  Ile  Tyr  Tyr  Tyr  Asn  Glu  Lys  Ala
25                      30                     35

Ile  Thr  Glu  Asn  Lys  Glu  Ser  Asp  Asp  Gln  Phe  Leu
               40                   45

Glu  Asn  Thr  Leu  Leu  Phe  Lys  Gly  Phe  Phe  Thr  Gly
     50                   55                          60

His  Pro  Trp  Tyr  Asn  Asp  Leu  Leu  Val  Asp  Leu  Gly
                    65                        70

Ser  Lys  Asp  Ala  Thr  Asn  Lys  Tyr  Lys  Gly  Lys  Lys
          75                   80

Val  Asp  Leu  Tyr  Gly  Ala  Tyr  Tyr  Gly  Tyr  Gln  Cys
85                      90                          95

Ala  Gly  Gly  Thr  Pro  Asn  Lys  Thr  Ala  Cys  Met  Tyr
               100                      105

Gly  Gly  Val  Thr  Leu  His  Asp  Asn  Asn  Arg  Leu  Thr
     110                      115                      120

     Glu  Glu  Lys  Lys  Val  Pro  Ile  Asn  Leu  Trp  Ile  Asp
                    125                      130
```

| Gly | Lys | Gln 135 | Thr | Thr | Val | Pro | Ile 140 | Asp | Lys | Val | Lys |

| Thr 145 | Ser | Lys | Lys | Glu | Val 150 | Thr | Val | Gln | Glu | Leu 155 | Asp |

| Leu | Gln | Ala | Arg 160 | His | Tyr | Leu | His | Gly 165 | Lys | Phe | Gly |

| Leu | Tyr 170 | Asn | Ser | Asp | Ser | Phe 175 | Gly | Gly | Lys | Val | Gln 180 |

| Arg | Gly | Leu | Ile | Val 185 | Phe | His | Ser | Ser | Glu 190 | Gly | Ser |

| Thr | Val | Ser 195 | Tyr | Asp | Leu | Phe | Asp 200 | Ala | Gln | Gly | Gln |

| Tyr 205 | Pro | Asp | Thr | Leu | Leu 210 | Arg | Ile | Tyr | Arg | Asp 215 | Asn |

| Lys | Thr | Ile | Asn 220 | Ser | Glu | Asn | Leu | His 225 | Ile | Asp | Leu |

| Tyr | Leu 230 | Tyr | Thr | Thr |

P A T E N T C L A I M S

1. A conjugate between
   a) a wild-type superantigen that has been modified, and
   b) a target seeking moiety;

wherein said modified superantigen comprises an amino acid
sequence of a first wild-type superantigen, in which one or
more amino acid residues in at least one region
   i)   which is present within said amino acid sequence
        of said first wild-type superantigen and
   ii) which functions in determining binding to TCR and
        activation of a subset of T cells have been replaced
by
        a different amino acid residue,
said modified superantigen retaining an ability to activate
a subset of T cells.

2. The conjugate of claim 1, wherein said at least one region
functions in determining binding to TCRVβ.

3. The conjugate of anyone of claims 1-2, wherein said wild-
type superantigen is SEA, SED, or SEE or analogous
superantigens.

4. The conjugate of anyone of claims 1-3, wherein said wild-
type superantigen is SEE and said at least one region is
selected from the group consisting of region A, C, F and H
as defined in Sequence ID no 8.

5. The conjugate of anyone of claims 1-4, wherein said wild-
type superantigen is SEE in which the following amino acid
residues substitutions have been made: R20G, N21T, S24G and
R27K where the positions are as defined in Sequence ID no 8
in figure 2.

6. The conjugate of anyone of claims 1-5, wherein the modified
superantigen is a chimera between

a) said first wild-type superantigen and

b) one or more additional wild-type superantigens,
wherein in each of said one or more amino acid residues in
said at least one region of said first wild-type
5   superantigen has been replaced with a corresponding amino
acid residue present in the corresponding region in said one
or more additional wild-type superantigens.

7. The conjugate of claim 6, wherein said first and additional
10   wild-type superantigens are selected from the group
consisting of SEA, SED, SEE and analogous superantigens.

8. The conjugate of claim 7, wherein said at least one region
is selected from the group consisting of regions
15   corresponding to A, C, F and H as defined in figure 2,
sequence ID nos 7 or 8.

9. The conjugate of claim 8, wherein at least one amino acid
residues at positions corresponding to 20, 21, 24 and 27 in
20   region A as defined in sequence ID nos 7 and 8, figure 2,
have been replaced with the corresponding amino acid
residues in region A of said one or more additional wild-
type superantigens.

25 10. The conjugate of anyone of claims 6-9, wherein said first
wild-type superantigen is SEE and said one or more
additional wild-type superantigen is SEA.

11. The conjugate of anyone of claims 7-10, wherein the
30   replacements of said one or more amino acids are at
positions corresponding to 20, 21, 24, 27 (for region A)
and/or 60, 62 (for region C) and/or 111, 114, 115, 117, 118,
124, 126 (for region F) and/or 200, 206, 207 (for region H)
as defined in figure 2, sequence ID nos 7 and 8, with
35   preference for the residue in all four positions in region A

superantigens, in the sense that each of said one or more
amino acid residues in said at least one region of said
first wild-type superantigen has been replaced with a
corresponding amino acid residue present in the
5 corresponding region of said one or more additional wild-
type superantigens.

17. The method of anyone of claims 15-16, wherein the disease
is associated with cells expressing a surface target
10 structure which bind to the superantigen at an epitope
structurally different from the epitope binding to TCR and
which binding to a surface binding structure allows for
binding to TCR with subsequent activation of a subset of T
cells.

15

18. The method of anyone of claims 15-17, wherein the disease
is selected from the group consisting of cancers, viral
infections, parasitic infestations and autoimmune diseases.

20 19. The method of anyone of claims 15-18, wherein said wild-
type superantigens are selected from the group consisting of
SEA, SED, SEE and analogous superantigens.

20. The method of claim 19, wherein said at least one region is
25 selected from the group consisting of regions corresponding
to regions A, C, F or H as defined in figure 2, Sequence ID
Nos 7 and 8.

21. The method of anyone of claims 15-20, wherein said first
30 wild-type superantigen is SEE and said at least one region
is selected from the group consisting of region A, C, F and
H as defined in Sequence ID no 8, figure 2.

22. The method of claim 21, wherein said at least one region is
35 region A and wherein the following amino acid residues
substitutions have been made: R20G, N21T, S24G and R27K

where the positions are as defined in Sequence ID no 8 in
figure 2.

23. The method of anyone of claims 15-22, wherein said at least
one additional wild-type superantigen is SEA.

24. The method of claim 15-23, wherein said superantigen is a
conjugate as defined in anyone of claims 1-14.

25. The method of claim 24, wherein the wild-type superantigens
have been selected among those that require binding to zinc
ions for binding to MHC Class II and that an amino acid
residue in the sequence of the first wild-type superantigen
has been replaced in a position involved in binding to zinc
in order to lower the MHC class II binding ability.

26. The method of claim 25, wherein said position corresponds
to 227 as defined in figure 2, sequence ID nos 7 and 8, with
emphasis of the mutation D227A.

27. The method according to anyone of claims 24-26, wherein the
target-seeking moiety is an Fab fragment in which each
cysteine residue providing interchain linkage has been
replaced with an amino acid residue not enabling interchain
linkage, with preference for serine being the replacing
amino acid residue.

28. A pharmaceutical composition comprising a modified antibody
in which cysteines providing for interchain cystine linkages
in the native antibody have been replaced to prohibit
cystine formation.

29. The pharmaceutical composition of claim 28, wherein the
replacing residue is a serine residue.

30. The pharmaceutical composition of claim 29, wherein the antibody is an Fab fragment.

31. The pharmaceutical composition of claim 30, wherein the antibody is fused to a peptide moiety providing activation of T-cells in a Vβ specific manner.
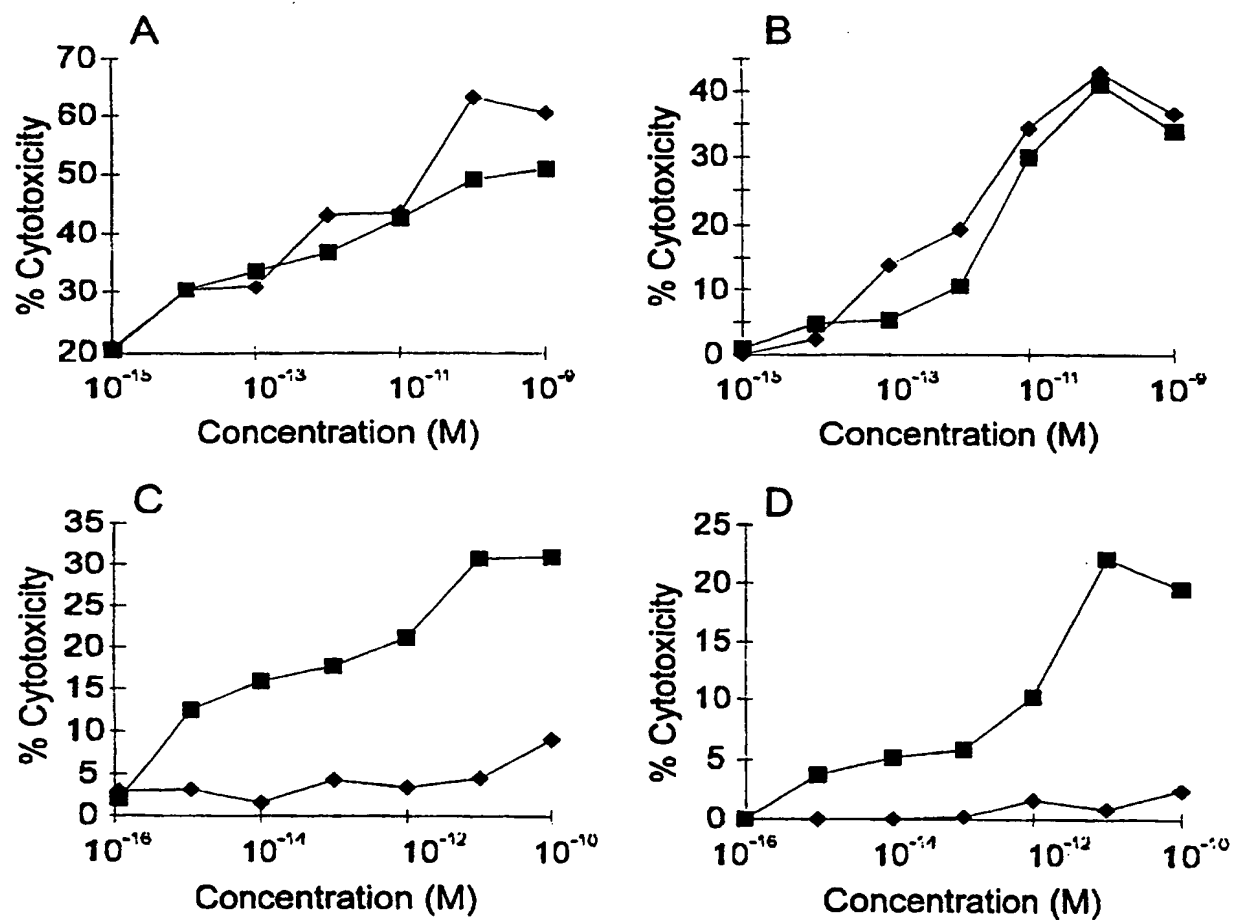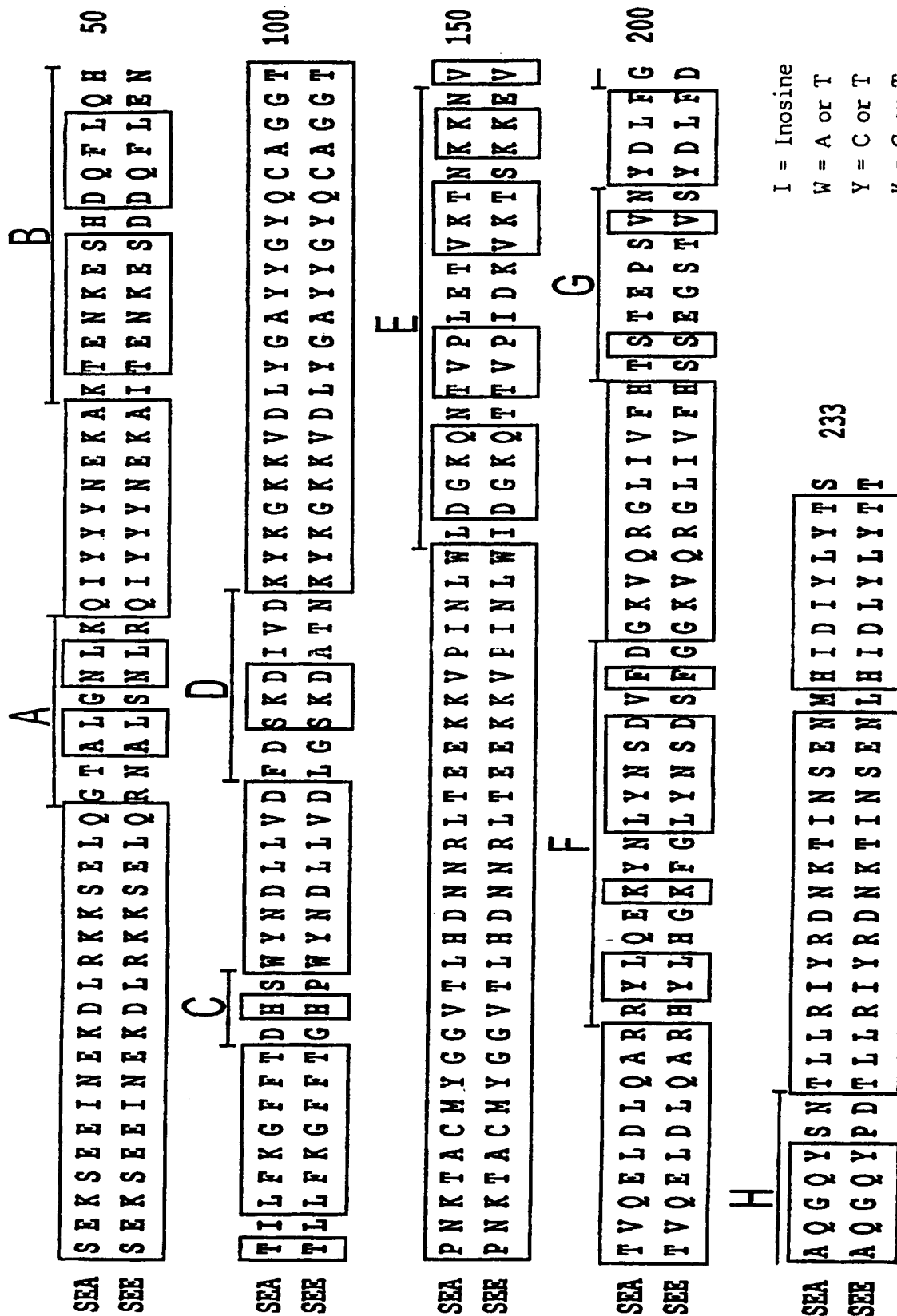
FIGURE 1

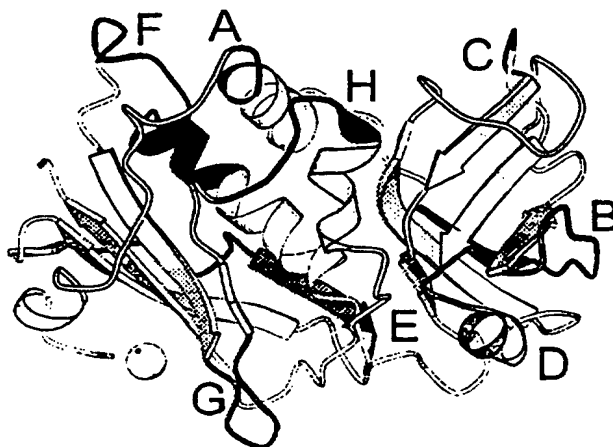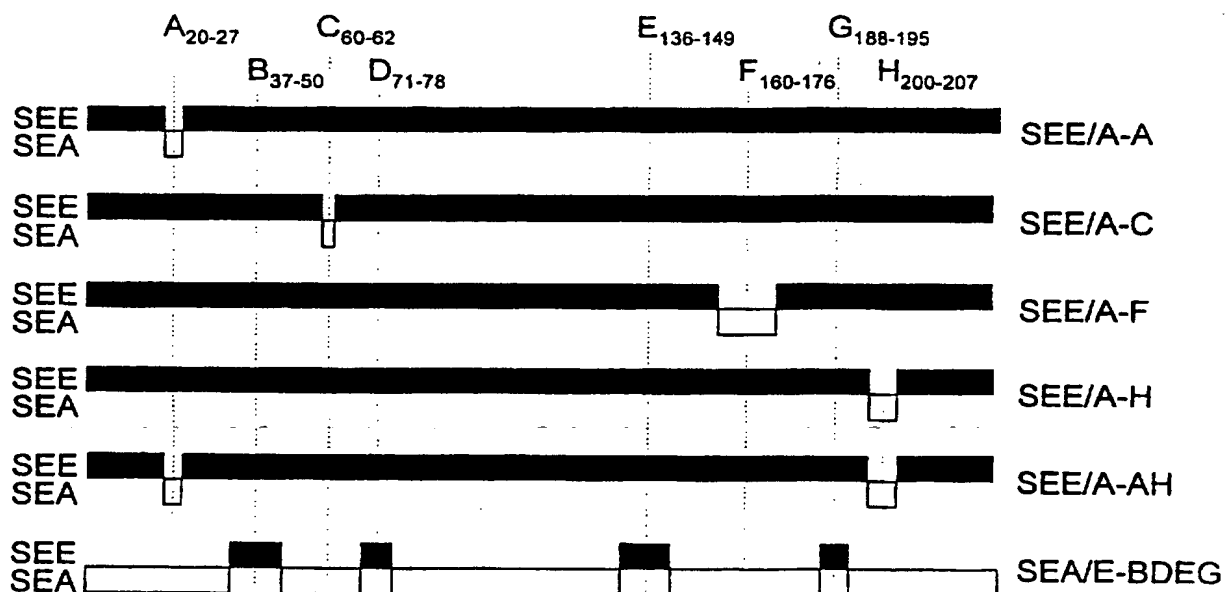Figure 2   SEQUENCE ID NO 7: SEA   SEQUENCE ID NO 8: SEE

I = Inosine
W = A or T
Y = C or T
K = G or T
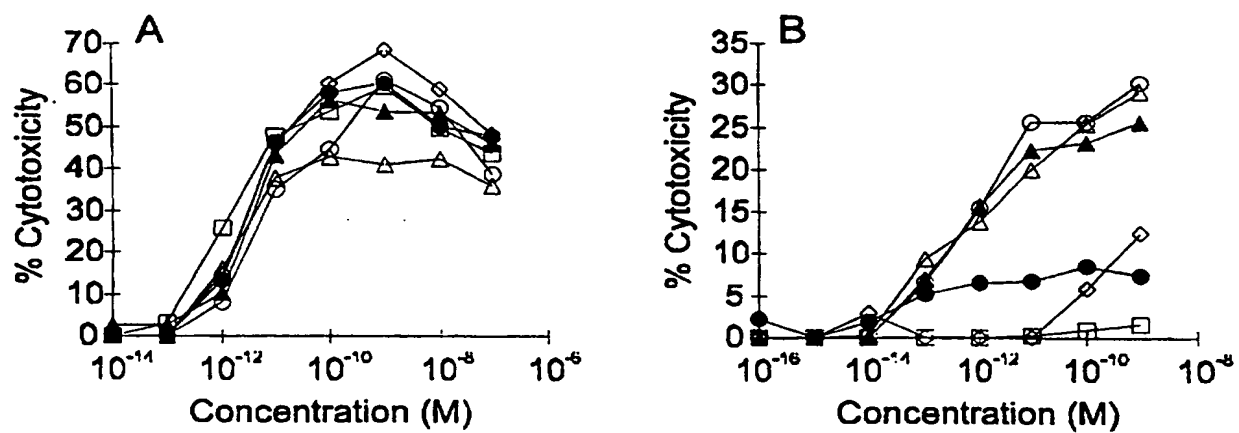
Figure 3.

Figure 4.

Figure 5.
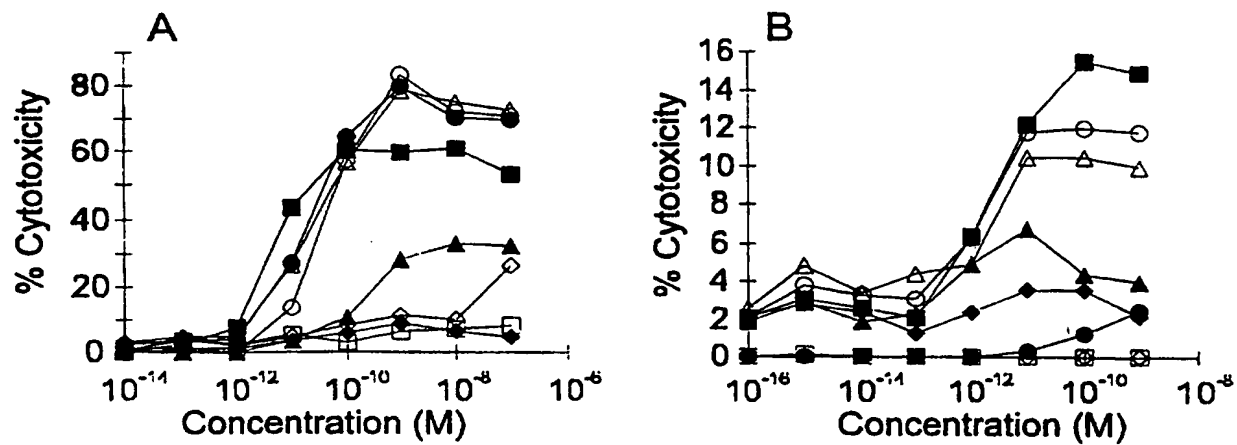
Figure 6.
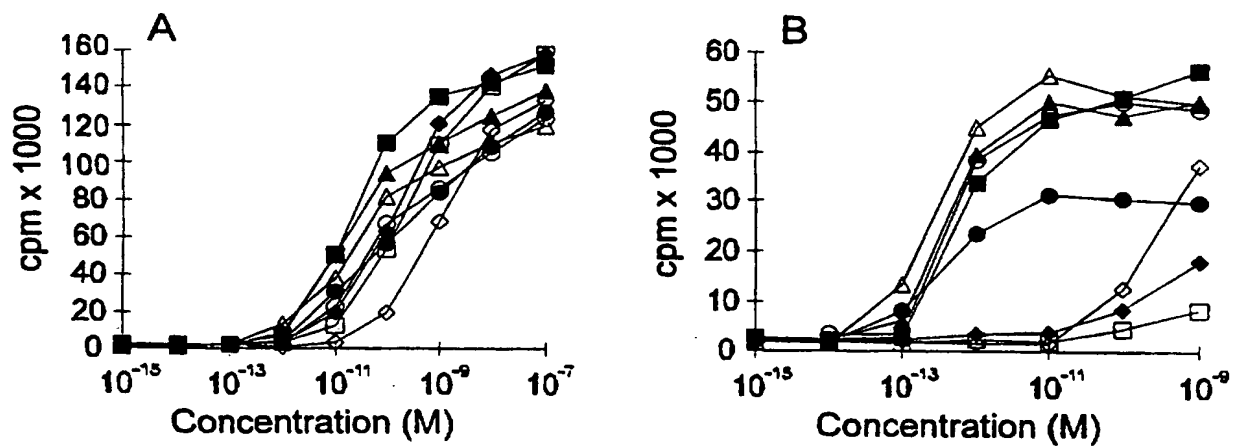
Figure 7.

Figure 8.
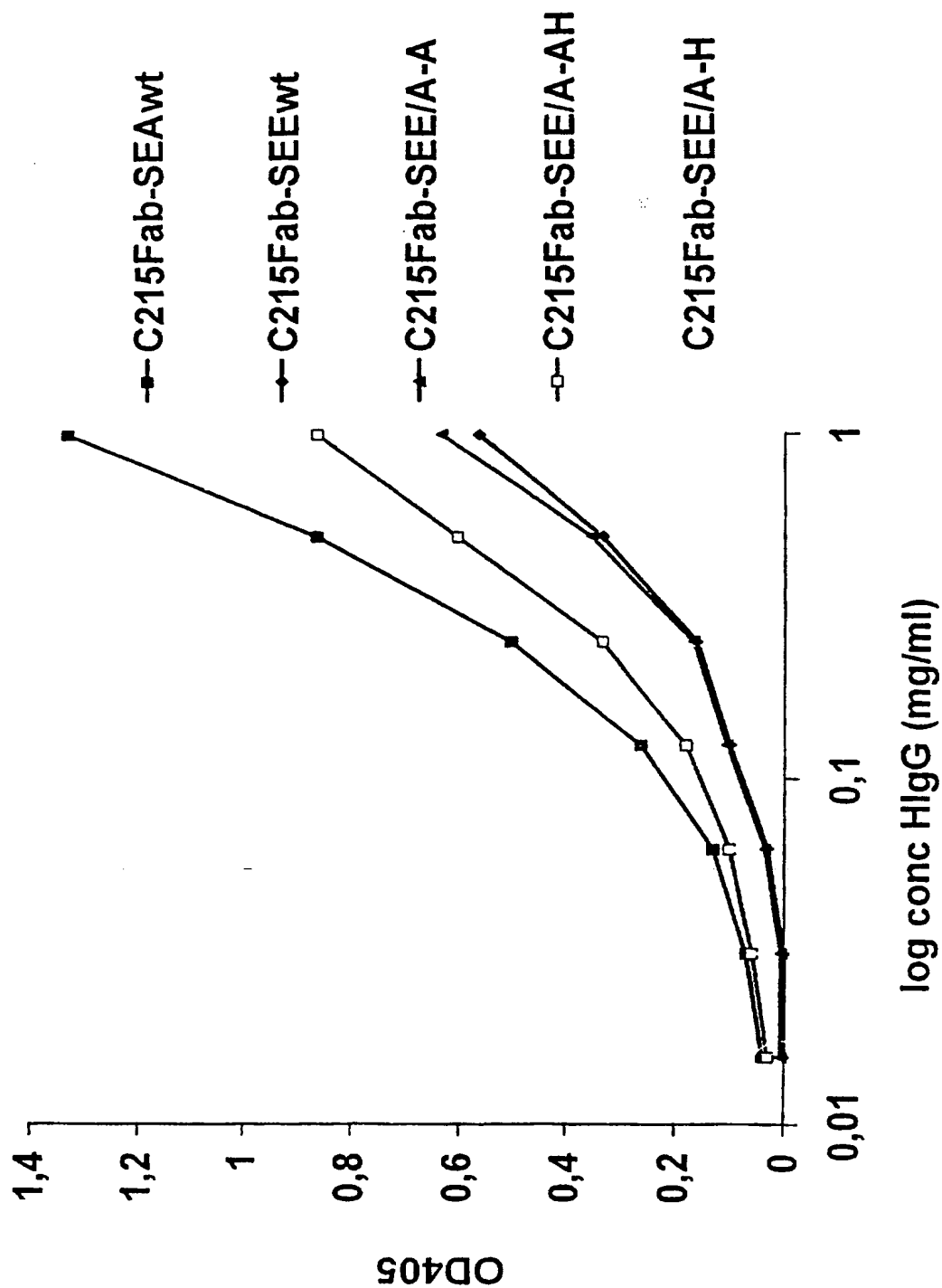
INTERNATIONAL SEARCH REPORT

International application No.	
PCT/SE 97/00537	

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 19/00, A61K 47/48, A61K 39/395, C07K 14/31
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SCISEARCH, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9601650 A1 (PHARMACIA AB), 25 January 1996 (25.01.96), see tables 1-3	1-26
A	J. Exp. Med., Volume 175, February 1992, John W. Kappler et al, "Mutations Defining Functional Regions of the Superantigen Staphylococcal Enterotoxin B", page 387 - page 396, see figure 2 and "Discussion"	1-11,13-25
A	J. Exp. Med., Volume 177, February 1993, Joseph A. Mollick et al, "Localization of a Site on Bacterial Superantigens That Determines T Cell Receptor beta Chain Specificity", page 283 - page 293, see table 1	1-11,13-25

☒ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	erlier document but published on or after the international filing date	"X"	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means	"Y"	document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 1997	27.08.97

Name and mailing address of the ISA/	Authorized officer
Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 97/00537

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Exp. Med., Volume 177, January 1993, Keith R. Hudson et al, "Two Adjacent Residues in Staphylococcal Enterotoxins A and E Determine T Cell Receptor Vbeta Specificity" page 175 - page 184	1-11,13-25
A	National Library of Medicine, file Medline, Medline accession no. 95136241, Ihle J: "Antibody-targeted superantigens induce lysis of major histocompatibility complex class II-negative T-cell leukemia lines", Cancer Res 1995 Feb 1;55(3):623-8	1-25
A	National Library of Medicine, file Medline, Medline accession no. 95385031, Holzer U et al: "Superantigen-staphylococcal- enterotoxin-A-dependent and antibody- targeted lysis of GD2-positive neuro- blastoma cells", Cancer Immunol Immunother 1995 Aug; 41(2):129-36	1-25
A	National Library of Medicine, file Medline, Medline accession no. 95347324, Abrahmsen L et al: "Characterization of two distinct MHC class II binding sites in the superantigen staphylococcal enterotoxin A", EMBO J 1995 Jul 3;14(13):2979-86	1-27
X	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 06620856, Medline accession no. 91355698, Gillies SD et al: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities"; Hum Antibodies Hybridomas 1990, 1 (1) p47-54	27-31
X	Proc. Natl. Acad. Sci., Immunology, Volume 90, January 1993, M. Better et al, "Potent anti-CD5 ricin A chain immunoconjugates from bacterially produced Fab' and F(ab')2" page 457 - page 461	27-31

INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 682039 A1 (BRISTOL-MYERS SQUIBB COMPANY), 15 November 1995 (15.11.95), see fig. 1 and example 2	27-31
A	WO 9601650 A1 (PHARMACIA AB), 25 January 1996 (25.01.96)	31
A	Dialog Information Services, file 34, SciSearch, Dialog accession no. 13524601, Mikolajczyk SD et al: "High-field, site-specific coupling of N-terminally modified beta-lactamase to a proteolytically derived single-sulfhydryl murine fab'", Bioconjugate Chemistry, 1994, V5, N6, P636-646	27,28,30
A	US 3903262 A (ALBERT R. PAPPENHAGEN ET AL), 2 Sept 1975 (02.09.75), see column 3-4 and claims	27
A	US 5541087 A (KIN-MING LO ET AL), 30 July 1996 (30.07.96), see column 10, lines 9-27	27

INTERNATIONAL SEARCH REPORT

Box I	Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 15-27
because they relate to subject matter not required to be searched by this Authority, namely:

   Claims 15-27 relates to a method of treatment of the human or animal body by surgery or by therapy/diagnostic method practised on the human or animal body/Rule 39.1(iv). Nevertheless, a search has been executed for this claims. The search has been based on the alleged effects of the conjugates.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II	Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

The present application refers to two a priori independent inventions:

1. Conjugates of a target seeking moiety and a modified wild type superantigen and a method of treating a disease with the aid of these conjugates, according to claims 1-26.

2. A pharmaceutical composition comprising an antibody lacking at least some cysteines that provide native interchain cysteine linkages, conjugates thereof and a method of treatment with such conjugates according to claims 27-31.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601650 A1	25/01/96	AU 2994095 A CA 2194673 A EP 0766566 A FI 970100 A IL 114445 D NO 970108 A PL 318162 A SE 9402430 A ZA 9505746 A	09/02/96 25/01/96 09/04/97 10/01/97 00/00/00 20/02/97 26/05/97 12/01/96 20/02/96
EP 682039 A1	15/11/95	NONE	
WO 9601650 A1	25/01/96	AU 2994095 A CA 2194673 A EP 0766566 A FI 970100 A IL 114445 D NO 970108 A PL 318162 A SE 9402430 A ZA 9505746 A	09/02/96 25/01/96 09/04/97 10/01/97 00/00/00 20/02/97 26/05/97 12/01/96 20/02/96
US 3903262 A	02/09/75	AT 321466 B CA 990647 A DE 2311333 A,C FR 2183679 A,B JP 1045762 C JP 48103723 A JP 55038931 B	10/04/75 08/06/76 27/09/73 21/12/73 28/05/81 26/12/73 07/10/80
US 5541087 A	30/07/96	AU 3676595 A CA 2199830 A EP 0782625 A WO 9608570 A WO 9608589 A	29/03/96 21/03/96 09/07/97 21/03/96 21/03/96